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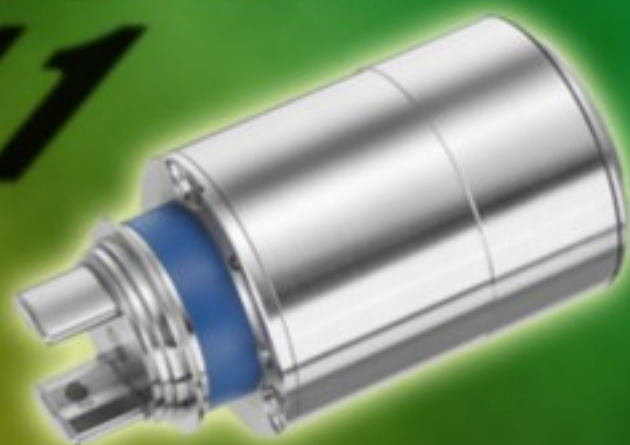
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A Bioelectrical Impedance Analysis Device for Monitoring Haemoglobin Status in Dengue Patients

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Abstract: The purpose of this study was to validate a single BIA for predicting hemoglobin (Hb) in dengue patients. The BIA technique based on the passing of low-amplitude electrical current less than 1 mA (500 to 800 μ A) with frequency 50 kHz. By using multiple regression analysis, reactance, sex, weight and vomiting were found independent determinants of predicting Hb. Hence, this novel approach of BIA technique can provide rapid, non-invasive, and promising method for monitoring and evaluating the status of the DHF patients. *Copyright © 2008 IFSA.*

Keywords: Bioelectrical impedance, Dengue fever, Dengue hemorrhagic, Hemoglobin, Multivariate analysis

1. Introduction

In 1970s the foundations of BIA were established, including those that underpinned the relationships between the impedance and the body water content of the body. The variety of single frequency BIA analyzers then became commercially available, and by the 1990s, the market included several multifrequency analyzers [1]. The use of BIA as a bedside method has increased because the equipment is portable and safe, the procedure is simple and non-invasive and the results are reproducible and rapidly obtained [1].

Many infectious diseases such as dengue, malaria, typhoid and hepatitis produce characteristic variations in the composition of blood [2]. These variations can be a characteristic change in number, size or shape of certain blood cells. For example, in anemia, the red blood cell (RBC) count is reduced [2]. Other diseases may cause changes in the chemical composition of the blood serum and other body fluid, like the urine. In diabetes mellitus, for instance, the glucose concentration in the blood and in urine is characteristically elevated in size and shape, or a chemical analysis of the blood serum can, therefore, provide important information for the diagnosis of such diseases [2]. Similarly, other body fluids, smears, and small samples of live tissue, obtained by biopsy, are studied through the technique of bacteriology, serology and histology to obtain clues for the diagnosis of diseases. However, these techniques are invasive because for the bacteriology, serology and histology diagnosis, require the sample of human's smear from the throat, blood and tissue respectively. The latest commercial technique takes two hours to detect dengue fever by serological confirmation using samples of serum, plasma or heparinized whole blood [2]. This test is still invasive and expensive and can only be performed by trained medical personnel.

Dengue fever (DF) and dengue haemorrhagic fever (DHF), ranks highly among the newly emerging infectious diseases in public health significance and is considered to be the most important of the arthropod-borne viral diseases. Since the early 1970s, the World Health Organization (WHO) has been actively involved in developing and promoting strategies for treatment and control of dengue. In 1997, WHO published a second guide to the diagnosis, treatment and control of dengue haemorrhagic fever [2]. Dengue were reported throughout the year and started to increase from 1997 to 1998. In 1998, 27,373 dengue cases with 58 deaths were reported as compared to 19,544 cases with 50 deaths in 1997. This has shown an increase of 7,829 cases or 40.1% over the number of cases in 1997 [3]. Therefore, there is an urgent need to design a system that can give early detection.

This paper describes a novel non-invasive approach to monitor the haemoglobin status in patients suffering from DHF by using bioelectrical impedance (single frequency) technique. In this study, we investigated the prognostic relevance of bioelectrical tissue conductivity (BETC) capacitive reactance (X_c), resistance (R), phase angle (α) and body capacitance (BC)) in BIA, to be the independent variables to monitor the haemoglobin status in DHF patients.

2. Bioelectrical Impedance Analysis Measurement

Human body can be represented as consisting of resistances and capacitances. In a healthy living body, the cell membrane consists of a layer of non-conductive lipid material sandwiched between two layers of conductive protein molecules. Biologically, the cell membrane functions as a permeable barrier separating the intracellular (cytoplasm) and extracellular components. It is traversed by numerous water soluble proteins, thus producing pores through which water, ions and other chemicals can enter and exit the cell. Controlling the flow of these materials is essential to life. The cell membrane protects the interior of the cell while allowing passage of some materials to which it is permeable.

The cell membrane is composed mostly of a double layer of phospholipids, arranged tail to tail along the width of the cell membrane. This structure is called the lipid bilayer and is an electrical insulator (dielectric), as all fats and oils are. The head of the phospholipids are polar (carry a charge) and the tails are non-polar. The heads interact with water, where the tail is repulsed by water aligning them tail to tail with the heads facing the outside and inside of the cell.

The structure of cell membranes makes them reactive, which behave as capacitors when exposed to an alternating current. Although total body water and extracellular water offer resistance to electrical current, only cell membranes offer capacitive reactance. Since fat tissue cells are not surrounded by cell membranes, reactance is not affected by the quantity of body fat.

Typical total body bioelectrical impedance (BIA) measurements display the vectors of resistance and reactance, which are intrinsically based on a series network of resistors and capacitors (Fig. 1).

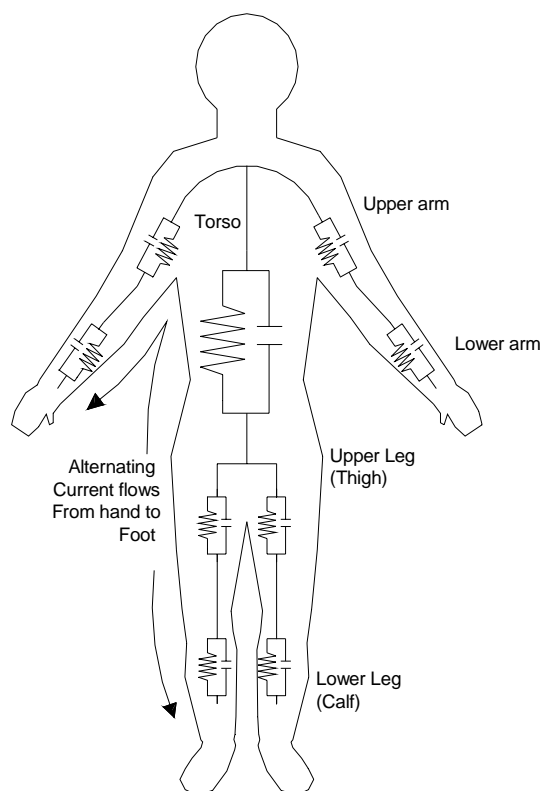


Fig. 1. Circuit of resistors and capacitors in the human body [4].

The resistance (R) of a length of homogeneous conductive material of uniform cross-sectional area, such as human body, is proportional to its length (L) and inversely proportional to its cross sectional area (A) as shown in Fig. 2. Although the body is not a uniform cylinder and its conductivity is not constant, an empirical relationship can be established between the impedance quotient (Length^2/R) and the volume of water, which contains electrolytes that conduct the electrical current through the body. Hence, the technique of BIA is based on the principle that the impedance, Z , (at given current frequency) of a cylindrical conducting body is related to the conductor length, L , resistivity, ρ , and conducting volume, V :

$$V = \rho L / Z^2 \quad (1)$$

The complexity is that the body offers two types of an electrical current, which are reactance and resistance. The capacitance arises from cell membranes, and the R from extracellular and intracellular fluid. Several electrical circuits have been used to describe the behavior of biological tissues in vivo [5].

In this study, the BIA measurements were conducted by way of a tetrapolar configuration [6] using the BIA 450 analyzer (Fig. 3). The four electrode technique used by this system largely avoided the aforementioned difficulties faced when using the two electrode technique. Four surface electrodes were used: two electrodes were placed on the subject's right hand, one at the base of knuckles and another slightly above the wrist joint. Another two electrodes were placed on the right foot, one near the base of the toes and the other slightly above the ankle joint.

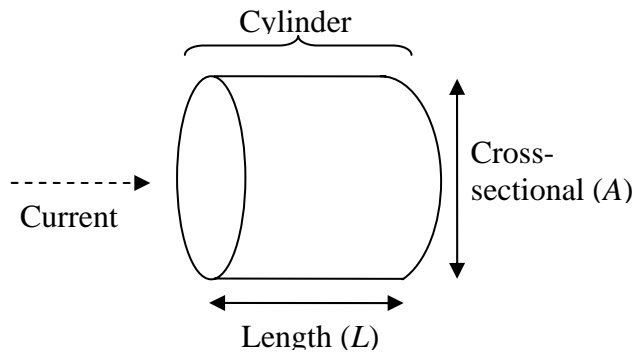


Fig. 2. Cylinder model for the relationship between impedance and geometry.

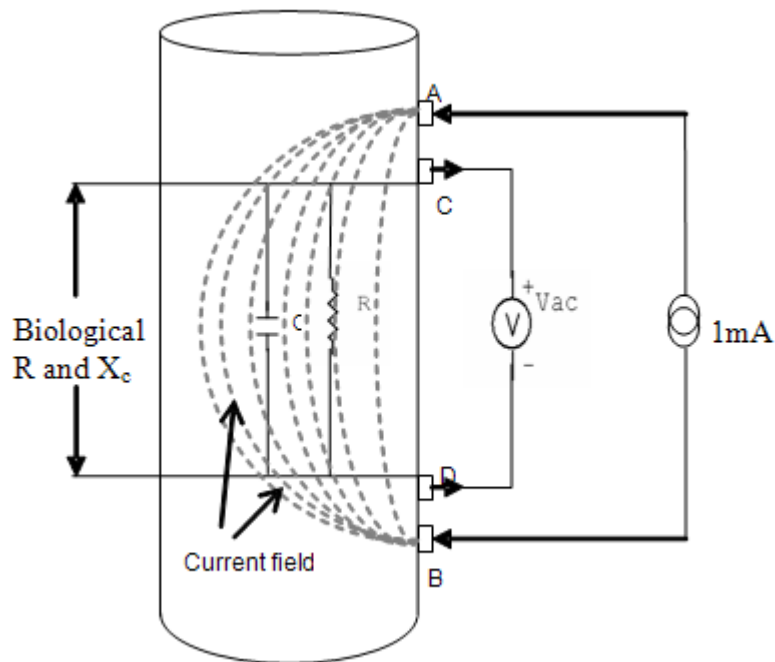


Fig. 3. Principle of bioelectrical impedance measurement using the four electrode technique. A and B are the current source electrodes (less than 1mA) while C and D are detecting electrodes.

The BIA 450 analyzer delivered constant current less than 1mA at 50 kHz into the tissue via the electrodes attached at base of the knuckles and base of the toes (current electrodes between points A and B) and the signal was picked up by the other two sensor electrodes (voltage electrodes between points C and D) slightly above the ankle and wrist joints as shown in Fig. 4.

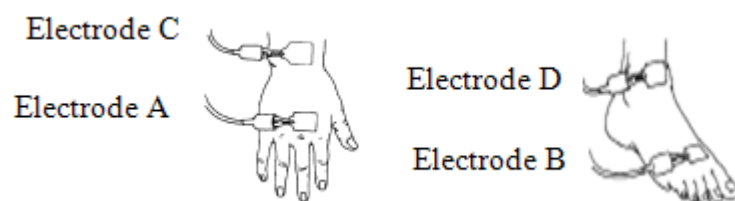


Fig. 4. Electrodes placement on the right side of the wrist and ankle. Electrodes A and B are current sources while electrodes C and D are voltage pick-up [7].

3. Statistical Analysis

Statistics were calculated with SPSS version 11.5, using non-parametric test because variables were not always normally distributed. Correlations between variables were analyzed using Spearman's rank correlation coefficient (ρ) and multiple linear regression analysis was used to determine the independent effect of parameters related with hemoglobin. Statistical significance was defined as $P < 0.05$ for all tests.

In multiple linear regression, each individual or case has scores on multiple independent variables (i.e. X_1, X_2, \dots, X_n if there is n independent variables) on the dependent variable (Y). A predicted dependent variable (Y) is formed that is a linear combination of the multiple independent variables. With n independent variables (predictors), the linear combination or multiple regression model equation is expressed as follows [8]:

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \dots + \beta_n X_{ni} + \varepsilon_i, \quad (2)$$

where

β_0 = Y -intercept or constant value;

β_1 = slope of Y with variable X_{1i} when variables $X_{2i}, X_{3i}, \dots, X_{ni}$ are held constant;

β_2 = slope of Y with variable X_{2i} when variables $X_{1i}, X_{3i}, \dots, X_{ni}$ are held constant;

β_3 = slope of Y with variable X_{3i} when variables $X_{1i}, X_{2i}, \dots, X_{ni}$ are held constant;

β_n = slope of Y with variable X_{ni} when variables $X_{1i}, X_{2i}, X_{3i}, \dots, X_{ni}$ are held constant;

ε_i = random error in Y for observation i .

The SPSS automatically computes a multiple correlation (r), a squared multiple correlation (R^2), and the adjusted squared multiple correlation (R^2_{adj}). All three indices assess how well the linear combination of predictor variables in the regression analysis predicts the criterion variable.

The residual analysis is then used to evaluate whether the multiple regression model is appropriate for the set of data being studied. The residual plot examines the pattern of residual for the predicted values of Y .

4. Patients and Methods

Two hundred ten adult patients aged 12 years old and above, suspected of DF and DHF admitted to the Universiti Kebangsaan Malaysia Hospital (HUKM), were monitored. The severity of the DHF is classified into grade I to IV, according to WHO recommendation [2]. Acute dengue infection was confirmed subsequently by the use of ELISA to detect elevated dengue specific IgM (primary infection) and IgG (secondary infection) [9]. Patient serum samples were tested for hemoglobin determination using an automated counter (Coulter STKS machine).

All patients were required to abstain from eating and drinking for 4h and from alcohol and physical exercise for 12h prior to the BIA measurements. The clinical data were recorded using the standardized questionnaire data collection from designated by Ibrahim et. al [10].

The patient's measurement was dated with referenced to day of fever settled when temperature dropped below 37.5°C . Fever day 0 was the day of fever settled. Day after fever settled is designated as day 1. The patients were asked to lie supine on their bed and each measurement took approximately 3 minutes. Two electrodes were placed on the patient's right hand, one the base of the knuckles and another slightly above the wrist joint. Another two electrodes were placed on the right foot, one near

the toes and the other slightly above the ankle joint. A constant current less than 1 mA and single frequency of 50 kHz was produced by a biodynamic Model 450 bioimpedance analyzer (Biodynamic Corporation, USA) and injected to the base of the knuckles and base of the toes and the signal was picked up by the other two sensor electrodes. Resistance, reactance, body capacitance and phase angle were measured by the BIA analyzer.

5. Results

Subjects were 210 patients, 119 males and 91 females with mean age of 30.65 years. Correlations between variables were analyzed using Spearman's correlation coefficient. It is a standardized measure of the strength of the relationship between two variables that does not rely on the assumptions of a parametric test. A matrix is displayed giving the correlation coefficient between the two variables such as gender and height (0.647), underneath is the significant values of the coefficient (0.000) and finally the sample size (210). The significant value for this correlation coefficient is less than 0.05. Therefore, it can be concluded that there is a significant relationship between a gender and height.

Linear regression was used to identify the most significant variable among the bioelectrical impedance analysis parameters. The significant variables were resistance and reactance ($p < 0.05$). Table 1 shows the model parameters. This model includes nine variables predicting the Hb, but only four variables are highly significant.

Table 1. Significant parameters for 210 dengue patients on day-of-admission.

Model	Coefficients (a)		Standardized Coefficients	t	Significance
	Unstandardized Coefficients				
	B	Standard Error	Beta		
(Constant)	6.012	3.75		1.603	0.112
GENDER	1.309	0.551	0.338	2.373	0.02
RISK	-0.241	0.32	-0.063	-0.753	0.453
HEIGHT	0.020	0.025	0.096	0.82	0.414
RACE	0.066	0.177	0.031	0.375	0.709
WEIGHT	0.029	0.014	0.264	2.059	0.042
RESISTANCE	-0.002	0.004	-0.105	-0.514	0.609
REACTANCE	0.047	0.019	0.327	2.48	0.015
VOMITING	1.178	0.493	0.191	2.388	0.019
ANOREXIA	0.156	0.341	0.035	0.458	0.648

a. Dependent Variable: Hemoglobin

The best model produced by the multilinear regression using four variables (gender, weight, reactance and vomiting) only yields an accuracy of 43%. This model can be written as follows:

$$Hb = 6.012 + 1.309 (\text{gender}) + 0.029 (\text{weight}) + 0.047 (\text{react}) + 0.19 (\text{vomiting}) + \varepsilon \quad (3)$$

where

gender = 0 for female and 1 for male;

weight = weight of patients in kg;

react. = reactance of patients in Ohm;
 vomiting = ejection of the stomach contents through the mouth;
 ε = error term.

Table 2 shows three outliers from the study, and all of the three cases were serologically confirmed secondary infection of dengue. Case numbers 134 and 159 were the patients who experienced the highest and second highest of Hb value out of the 210 patients while case number 35 was the one who experienced the lowest Hb. These patients stayed in the hospital for 4.6 days (average length) and survived.

Table 2. Case wise Diagnostics for haemoglobin modeling.

Case number	Hemoglobin	Predicted value	Residual
35	6.7	11.303	-4.603
134	18.7	14.339	4.361
159	18.6	15.727	2.873

The examination of the assumptions of homogeneous variance, linearity and normality was done by residual analysis. Examining the scatter plot of standardized residuals against Hb (Fig. 5), it was found that the assumptions of linearity and homogeneous variance were met. Furthermore, the points on the normal probability plot (Fig. 6) sit on the diagonal line, suggesting that the normality assumption was not violated.

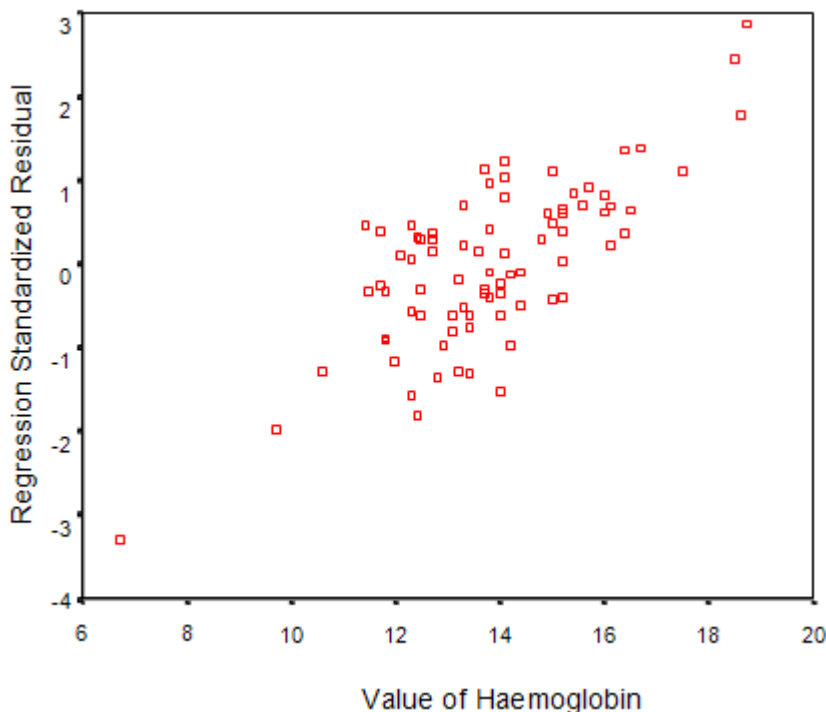


Fig. 5. Scatter plot of standardized residuals against haemoglobin.

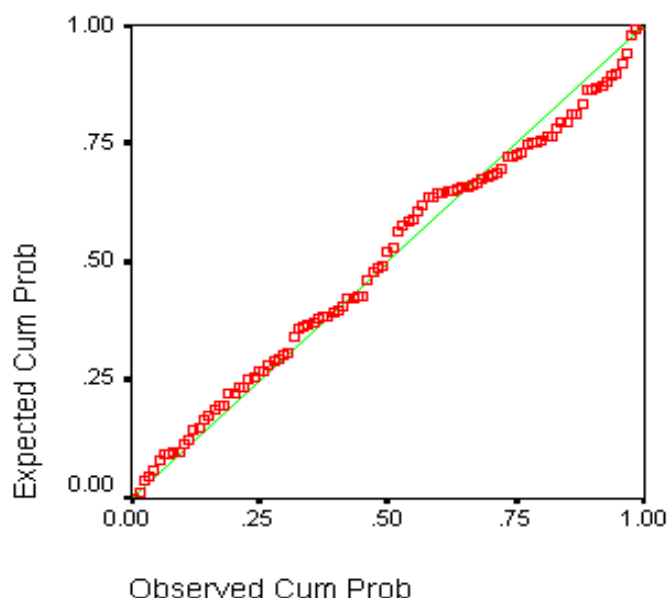


Fig. 6. Normal P-P plot of regression standardized residuals.

6. Validation of Statistical Analysis

The actual Hb values were compared with the Hb model obtained as can be seen in Table 3. The calculated values from the Hb model match very well with the actual Hb values obtained from the blood sample. For example, a female patient with reactance of 77.70 Ω , weight of 38.00 kg and with sign of vomit had Hb of 12.30 g/dl based on her blood sample, *c.f.* 12.31 g/dl evaluated by the model. Case number 15, female patient with reactance of 52.90 Ω , weight of 70.00 kg and with no sign of vomit had Hb of 12.70 g/dl based on her blood sample, *c.f.* 12.56 g/dl evaluated by this model. Another example for male patient with reactance of 57.50 Ω , weight of 54.00 kg and with no sign of vomit had his Hb measured at 14.00 g/dl, *c.f.* 13.97 g/dl when evaluated using the Hb model. Case number 199, with reactance of 50.20 Ω , weight of 76.00 kg and with sign of vomit had Hb of 16.50 g/dl based on her blood sample, *c.f.* 15.22 g/dl evaluated by this model.

Table 3. Comparison between actual and predicted hemoglobin according to gender.

Case number	Gender	Weight (kg)	Reactance (Ω)	Vomiting	Actual hemoglobin (g/dl)	Predicted Hemoglobin (g/dl)	Residual (g/dl)
2	Female	38.00	77.70	1	12.30	12.31	0.01
15	Female	70.00	52.90	0	12.70	12.56	0.14
194	Male	54.00	57.50	0	14.00	13.97	0.03
199	Male	76.00	50.20	1	16.50	15.22	1.28

7. Conclusion

A novel approach to classify DHF patients using BIA has been described. The findings show that reactance (X_c) was found to be a potentially useful tool in classifying the risk factor of DHF patients. New non-invasive system to predicting Hb in DF and DHF was developed.

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